

Bio-based Control for Invasive Mussels

Prepared for California State Water Resources Control Board July 24, 2014









Forward Looking Statement



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Topics of Discussion



- California Invasive Mussel Challenge
- About Zequanox
 - Product History and Development
 - Zequanox Treatment Programs
- Regulatory Status
- Main Points of Clarification for DPR
- General Permit Addition Request



Invasive Mussels Cause \$ Billions in Economic & Environmental Damage





Vulnerability within California









Why Bio?



- Safer alternatives to standard chemicals to protect public health and the environment
- Well established technology in the pharmaceutical and agricultural industries
 - Currently about 11% of pesticides and >50% of human drugs are derived from natural products
 - Bacillus thuringiensis (israelensis) used to control black fly larva in open waters - globally
 - Other *Pseudomonas* species are registered for plant health and frost protection in the US and Canada

Often highly selective/target specific

Safer for workers

Delayed onset of resistance

Better public perception



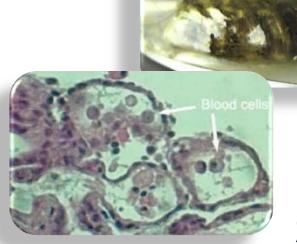


Zequanox: The Industry's Only Biopesticide for Invasive Mussel Control



- Environmentally friendly
 - Derived from soil microbe (Pseudomonas fluorescens) discovered by NYSM
 - Composed of 100% dead cells
- Controls mussels in all life stages
 - Perceived as food source—destroys the mussel's digestive system
- Highly selective toward zebra/quagga mussels
- Effective in a broad range of water conditions and temperatures
- Noncorrosive to infrastructure and equipment
- Nonvolatile





Siphon

Zequanox Treatment Programs



- Product applied with standard equipment
- Treatments can be completed within hours
 - Up to 8hrs depending on program
- Mortality occurs over time, reducing damage to equipment from shell debris
- Safe for employees in the surrounding area
- Mortality typically monitored via biobox systems

Annual

Designed for facilities with tolerance for moderate to large shell sizes (larger than 4 mm in size)

Treatments occur annually; typically end of season

Bi-Weekly

Goal is to limit the number of mussels that exceed 4 mm in size (adult sized)

Ideal for sensitive systems and equipment

Treatments are performed every other week throughout the settlement season



Regulatory Status



- US Environmental Protection Agency approves commercial formulation (2012)
 - Food tolerance exempt (76 FR 52871)
 - Current CA label covers enclosed systems
 - Open water label EPA approved June 2014
- Applied for multiple state registrations
 - Currently registered in 25 states, including NY and CA
- Canada's Pest Management Regulatory Agency approved commercial formulation in 2012
 - Current label covers hydropower applications
 - Application for label expansion in process
- European Union Dossier submitted Dec 2012 for Annex I inclusion



Main Points of Clarification for DPR



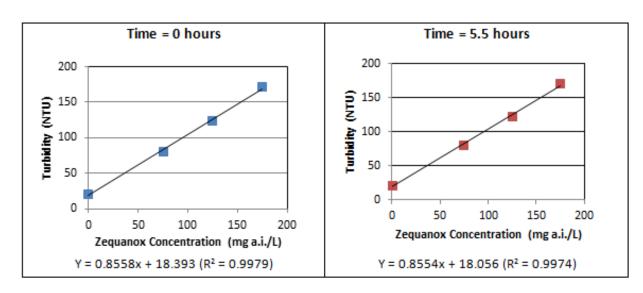
- Accuracy of using turbidity as a surrogate to measure product concentration in naturally turbid waters.
- Selection of appropriate modeling scenarios to evaluate worst-case environmental, fish, and wildlife impacts in California.
- Understanding the applicability of non-target toxicity testing for evaluating potential impacts to non-target wildlife.



Accuracy of Zequanox Measurement in Natural Waters



- Provided DPR with a Standard Operating Procedure on how turbidity could be used to measure Zequanox concentrations under field conditions.
- Clarified that methodology accounts for background turbidity and suspended solids at individual sampling times and site locations.
- Provided example measurement data in natural waters. Example data were also shared with the Board to support monitoring in receiving waters.
- Example linear regression showing correlation between turbidity and Zequanox concentration in Mississippi River in water at two different times shown below.





Modeling Worst-Case Discharge for California (



- Environmental and Fish and Wildlife reviewers challenged in conducting reviews of broad use patterns for Zequanox
- Discussed low-dilution modeling scenarios do not correspond to commercially viable or realistic use scenarios.
- Label application rates and application times lowered below EPA approved levels
 - 12 hour lowered to 8 hour application time
 - 200 lowered to 100 milligrams active ingredient per liter application concentration
- Low dilution uses would be further addressed by the State
 Water Resources Control Board NPDES permitting process



Applicability of Non-target Studies



- Non-target toxicity testing conducted according to guidelines.
 Studies encompassed 24 96 hour exposure periods.
 - Select studies incorporated media renewal to account for potential degradation of Zequanox over the study period.
- Additional studies requested by DPR were provided to facilitate evaluation of potential impacts on select species.
 - Key 96-hour fish studies were conducted using a flow-through study design where exposure concentrations could be determined via turbidity measurements.
- Provided information to increase confidence in the testing exposure concentrations, and derived LC₅₀ values, allowing DPR to reliably evaluate the potential for non-target impacts.



Request for General Permit Addition



- Zequanox received registration in California November 2013
- Interested customers have specifically requested general permit addition
- San Diego County Water Authority hoping to conclude treatment alternatives evaluation by end of summer of 2014
- Expedited SWRCB review requested by both MBI and SDCWA.





Request for Amendment to General Permit Supporting Documentation



- Suggested General Permit Language
 - Proposed receiving water limitations and monitoring requirements
 - Proposed general editorial language to describe the product within the general permit framework
- Non Target Toxicity Overview for Closed Systems
- Drinking Water Contaminant Testing
 - Completed evaluation to understand potential impacts to water quality



Proposed Receiving Water Limitation and Monitoring Requirements



- Daily Maximum Concentration in Receiving Waters
 - 6 mg active ingredient/L (mg a.i./L)
 - Based on 1/10th lowest acute LC₅₀ for *Oncorhynchus mykiss* [rainbow trout] 96-hr LC_s= 59
 - Most conservative number, a higher quality study with rainbow trout resulted in LC50 greater than 100 mg a.i./L - described in ecotoxicity overview
- Concentration monitored through site specific correlation between turbidity and the product active ingredient
 - MBI provided data to the Board to demonstrate effectiveness and practice of using turbidity monitoring



Proposed Limitation



Constituent	Limitation	Basis		
Chlorine	10 ug/l – Monthly Average	U.S. EPA's Ambient Water Quality Criteria for Freshwater Aquatic Life Protection		
Chlorine	20 ug/l – Daily Maximum	U.S. EPA's Ambient Water Quality Criteria for Freshwater Aquatic Life Protection		
Chlorine	<10 ug/l – Daily Maximum	California Ocean Plan		
Pseudomonas fluorescens strain CL145A cells and spent fermentation media	Daily Maximum - 6 mg a.i./L	One-tenth of the lowest LC_{50} value: Oncorhynchus mykiss [rainbow trout] 96-hr LC_{50} = 59.09 ^a		

^a Hartwell, T. (2011) Rainbow Trout (Oncorhynchus mykiss) 96-Hour Acute Toxicity Test. Study No.: 14732-10.

(Stillmeadow, Inc., Sugar Land, TX)

Proposed Monitoring



Sample Type	Constituent/Parameter	Units	Sample Method	Minimum Sampling Frequency	Sample Type Requirement	Required Analytical Test Method
Visual	 Monitoring area description (pond, lake, open waterway, channel, etc.) Appearance of waterway (sheen, color, clarity, etc.) Weather conditions (fog, rain, wind, etc.) 	Not applicable	Visual Observation	1	Background, Event, and Post-Event Monitoring	Not applicable
Physical	 Temperature² pH³ Turbidity³ Electrical Conductivity³ @ 25°C 	°F Number NTU µmhos/cm	Grab ⁴ <u>or In</u> Situ water quality probe ⁹	5	Background, Event, and Post-Event Monitoring	6
Chemical	 Chlorine Pseudomonas fluorescens strain CL145A cells and spent fermentation media⁷ Dissolved Oxygen³ 	μg/L <u>mg a.i./L⁸</u> mg/L	Grab ⁴ <u>or In</u> Situ water quality probe ⁹	5	Background, Event, and Post-Event Monitoring	6 <u>/7</u>

Non Target Toxicity Overview for Closed Systems



 Intended to present full picture and contextualize the wide body of studies according to product usage



- Confidential full reports available for all studies
- Extensive testing on native unionids
- Comparison of available data to Whole Effluent Toxicity Testing Guidelines













Drinking Water Contaminant Testing



- Tested regulated drinking water contaminates at 1 and 10 mg a.i./L Zequanox
 - Above and below proposed receiving water limitation
- Few regulated drinking water contaminants were detected
- Aluminum was detected at 0.2 mg/L in the 10 mg a.i./L ZQ dilution, below the CA MCL of 1 mg/L
 - National secondary standard is 0.2 mg/L
 - Aluminum will likely precipitate out in the environment or be removed during conventional treatment because it is an inert ingredient present in a particulate form
 - No other constituents were detected at or above MCLs or Secondary Standards.



Questions?



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